Patent claims

1. Process for the presentation of peptides or/and polypeptides on the surface of Gram-negative host bacteria, where

a) there is provision of a host bacterium which [lacuna] transformed with a vector on which is located, operatively linked to a promoter, a fused nucleic acid sequence comprising:

(i) a signal peptide-encoding nucleic acid section,

(ii) a nucleic acid section coding for the passenger peptide or/and passenger polypeptide to be presented,

(iii) where appropriate a nucleic acid section coding for a protease recognition site,

(iv) a nucleic acid section coding for a transmembrane linker and

(v) a nucleic acid section coding for a transporter domain of an autotransporter; and

(b) the host bacterium is cultivated under conditions with which there is expression of the fused nucleic acid sequence and presentation of the peptide or polypeptide encoded by the nucleic acid section (ii) on the surface of the host bacterium,

characterized in that the nucleic acid section (ii) is heterologous in relation to the nucleic acid section coding for the transporter domain (v), and the host bacterium is homologous in relation to the nucleic acid section coding for the transporter domain (v).

2. Process according to Claim 1, characterized in that the autotransporter used has been derived from a genus of enterobacterioceae and is used in a host bacterium of a genus of enterobacterioceae.

3. Process according to Claim 1 ~~or 2,~~ characterized in that the transporter domain of the Aida protein from E.coli or a variant thereof is used.

4. Process according to Claim 1 ~~or 2~~, characterized in that the transporter domain of the SepA protein from Shigella flexneri or a variant thereof is used.

5. Process according to Claim 1 ~~or 2~~, characterized in that the transporter domain of the IcsA protein from Shigella flexneri or a variant thereof is used.

6. Process according to Claim 2, characterized in that the transporter domain of the Tsh protein from E.coli or a variant thereof is used.

7. Process according to Claim 2, characterized in that the transporter domain of the Ssp protein from Seratin marcescens or a variant thereof is used.

8. Process according to Claim 1, characterized in that the transporter domain of the Hsr protein from Helicobacter mustelae, of the Prn protein from Bordetella ssp., of the Hap protein from Haemophilus influenzae, of the BrkA protein from Bordetella pertussis, of the VacA protein from Helicobacter pylori or of one of the rickettsial proteins 190kDa cell surface protein, SpaP, rOmpB or SlpT, is used.

9. Process according to ~~any of Claims 1-8~~ Claim 1, characterized in that one or more peptides, in particular peptides having a length of 4-50 amino acids, are presented.

10. Process according to ~~any of Claims 1-8~~ Claim 1, characterized in that one or more eukaryotic polypeptides are presented.

11. Process according to Claim 10, characterized in that the passenger polypeptide is an antibody or an antigen-binding domain of an antibody, where antigen-binding domain refers to at least the region of an antibody molecule which is sufficient for specific binding of an antigen.

12. Process according to Claim 10, characterized in that the passenger polypeptide is the α chain of an MHC class II molecule.

13. Process according to Claim 10, characterized in that the passenger polypeptide is the β chain of an MHC class II molecule.

14. Process according to Claim 13, characterized in that the passenger polypeptide is the β chain of an MHC class II molecule, attached to whose N terminus are amino acids which, as peptide, are able to embed in the binding cavity of the functional MHC molecule.

15. Process according to ~~any of Claims 1-14~~ claim 1, characterized in that libraries of variant passenger peptides or polypeptides are produced, expressed in host cells and presented on the surface.

16. Process according to Claim 15, characterized in that the variant passenger peptides or polypeptides are presented in a constant context of a passenger polypeptide.

17. Process according to ~~any of Claims 1-16~~ claim 1, characterized in that a host bacterial cell presents different passenger peptides or polypeptides in each case connected to a transporter domain.

18. Process according to Claim 17, characterized in that different transporter domains are used in connection with different passenger peptides or polypeptides.

19. Process according to ~~any of Claims 15-18~~ claim 15, further comprising the step of selecting single passenger peptides or polypeptides from a library of variant peptides or polypeptides.

20. Process for the preparation of a variant population of surface-exposed peptides or polypeptides and for identification of the bacteria which carry peptides or polypeptides with a particular required property, where the process comprises the following steps:

(1) preparation of one or more fusion genes by cloning the coding sequence of a required passenger in frame with the coding sequence of the transporter domain of an autotransporter and of a signal peptide in at least one vector;

(2) variation of the passenger peptide or polypeptide by mutagenesis;

(3) introduction of the vector or vectors into host bacteria able to present the passenger or passengers stably on the surface;

(4) expression of the fusion gene or fusion genes in the host bacteria;

(5) cultivation of the bacteria to produce the passenger presented stably exposed on the surface or the passengers presented stably exposed on the surface;

(6) where appropriate selection of the bacteria which carry the passenger or passengers having the required properties on the surface, and

(7) where appropriate characterization of a binding partner for the passenger having the optimal properties.

21. Process according to Claim 20, where individual steps of the process can be omitted.

22. Process according to Claim 20, where the process is performed several times.

23. Process according to Claim 20, where the transporter domain ~~AIDA-I~~ or a variant thereof is used.

24. Process according to any of Claims 20-23, where the passenger protein present in the fusion protein is a peptide or polypeptide having an affinity for a binding partner, or is a ligand, a receptor, an antigen, a toxin-binding protein, a protein with enzymatic activity, a nucleic acid-binding protein, an inhibitor, a protein having chelator properties, an antibody or an antigen-binding domain of an antibody.

25. Process according to any of ~~Claims 20-24~~ claim, where the bacterium which presents a surface-exposed

passenger having a required binding affinity is identified by binding to an immobilized or/and labelled binding partner.

26. Process according to Claim 20, where the binding partner is modified so that it can be detected in a second step by a binding partner specific for the modification.

27. Process according to any of Claims 1-26, characterized in that passenger proteins or parts thereof are chemically or enzymatically modified on the bacterial surface.

28. Process according to Claim 27, characterized in that the modification is a non-covalent modification.

29. Process according to Claim 27, characterized in that the modification is a covalent modification.

30. Process according to Claim 29, characterized in that the modification is a glycosylation.

31. Process according to Claim 29, characterized in that the modification is a phosphorylation.

32. Process according to Claim 27, characterized in that the modification is a proteolysis.

33. Process according to Claim 32, characterized in that passenger proteins or parts thereof are selectively released from the bacterial surface by intrinsic or externally added proteases.

34. Process according to Claim 33, characterized in that passenger proteins or parts thereof are released by an intrinsic protease of the host cell, in particular OmpT protease, OmpK protease or protease X.

35. Process according to Claim 33, characterized in that passenger proteins or parts thereof are released by an externally added protease, in particular IgA protease, thrombin or factor X.

36. Recombinant vector on which is located, operatively linked to a promoter, a fused nucleic acid sequence comprising:

(i) a signal peptide-encoding nucleic acid section,

(ii) a nucleic acid section coding for the passenger peptide or/and passenger polypeptide to be presented,

(iii) where appropriate a nucleic acid section coding for a protease recognition site,

(iv) a nucleic acid section coding for a transmembrane linker and

(v) a nucleic acid section coding for a transporter domain of an autotransporter;

where the nucleic acid section (ii) is heterologous in relation to the nucleic acid section coding for the transporter domain (v).

37. Recombinant Gram-negative host bacterium, characterized in that it is transformed with a vector according to Claim 36.

38. Host bacterium according to Claim 37, characterized in that it is homologous in relation to the nucleic acid section coding for the transporter domain (v).

39. Host bacterium according to Claim 38, characterized in that it is an E.coli cell.

40. Host bacterium according to any of ~~Claims 37-39~~, characterized in that the nucleic acid section (y) codes for the transporter domain of the AIDA protein or a variant thereof.